AD-A166 523 INTRINSIC ALTERATION OF THE REACTIVE PROPERTIES OF ARTERIES DURING HYPOTHERMIA(U) ARMY RESEARCH INST OF ENVIRONMENTAL MEDICINE NATICK MA N R BANDICK ET AL. 1/1

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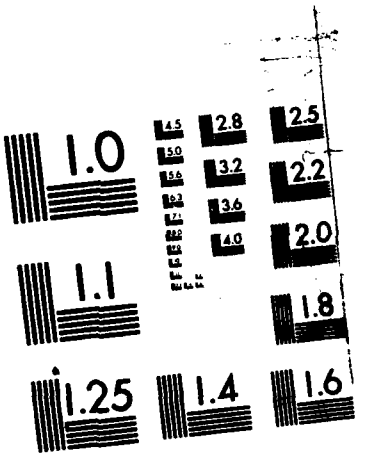

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Norepinphrine sensitivity; whole body hypothermia; vascular smooth muscle; receptors; rabbits

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This study compared the reactive and contractile properties of helical strips of femoral arteries taken from normothermic rabbits and rabbits that were anesthetized with pentobarbitol and cooled to 25°C at a rate of 7°C per hour. The purpose of this comparison was to see if intrinsic factors would alter the sensitivity and/or contractility of this vascular muscle to norepiniphrine during whole body hypothermia. We found that, after two hours of in vivo hypothermia, the hypothermic derived tissue was from 10 to 100 times more

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sensitive to norepiniphrine than the normothermic derived tissue. This augmented sensitivity continued while the tissue was in vitro for at least twelve hours. The dose-response curves of the hypothermic derived arteries were shifted to the left of the normothermic arteries resulting in a greater contractility at lower levels of agonist. Moreover, the normothermic tissue contracted slower than the hypothermic. The maximal tension developed by the strips was equivalent. This study has identified prolonged alterations of receptor sensitivities and contractility properties attributable to in vivo influences than can, in part, explain the disruptions of blood pressure during and following whole body hypothermia.

INTRINSIC ALTERATION OF THE REACTIVE PROPERTIES OF ARTERIES DURING HYPOTHERMIA

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Running title: Posthypothermic vessel action

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LIST OF FIGURES

Abstract

This study compared the reactive and contractile properties of helical strips of femoral arteries taken from normothermic rabbits and rabbits that were anesthetized with pentobarbitol and cooled to 25°C at a rate of 7°C per hour. The purpose of this comparison was to see if intrinsic factors would alter the sensitivity and/or contractility of this vascular muscle to norepinephrine during whole body hypothermia. We found that, after two hours of in vivo hypothermia, the hypothermic derived tissue was from 10 to 100 times more sensitive to norepinephrine than the normothermic derived tissue. This augmented sensitivity continued while the tissue was in vitro for at least twelve hours. The dose-response curves of the hypothermic derived arteries were shifted to the left of the normothermic arteries resulting in a greater contractility at lower levels of agonist. Moreover, the normothermic tissue contracted slower than the hypothermic. The maximal tension developed by the strips was equivalent. This study has identified prolonged alterations of receptor sensitivities and contractility properties attributable to in vivo influences than can, in part, explain the disruptions of blood pressure during and following whole body hypothermia.

Norepinphrine sensitivity; whole body hypothermia; vascular smooth muscle; receptors; rabbits.

Introduction

Humans that have recovered from whole body hypothermia typically have alternating episodes of hypertension and hypotension. The time of this cardiovascular instability can last beyond 48 hours and make it difficult for suitable patient care. The hypertension is achieved even though there is an augmented venous compliance and reduced cardiac output during this time. Therefore, the spasmodic raising and lowering of systemic arterial pressure must be attributed to changes in the total peripheral resistance through alterations of the arteriolar radius. Suspect components, within a negative feedback system of arteriolar control, would be the activity of arterial baroreceptors, neural cardiovascular control centers, plus the senstivity and/or contractility of the vascular smooth muscle. Most of the information on the role of blood vessel walls in this situation has come from observations during local cooling (1,5,6,7,10), on isolated perfused vessels (12,13,17), and on strips of blood vessel walls placed in a muscle bath (2,15,16). These latter studies have taken blood vessels from normothermic animals and subjected them to cooling in a bath to determine the alterations to contractility and reactivity. These methods have provided information on the in vitro effects of temperature on isolated blood vessel wall but have not permitted observations on in vivo mechanisms and influences during whole body hypothermia, particularly those that would persist for greater than 48 hours after the subject has been warmed to normothermic levels. The intent of this study was to determine if there were differences between in vitro and in vivo hypothermic alterations of the contractility and reactivity of blood vessel walls. If in vivo mechanisms could be identified and appropriately manipulated, then perhaps more suitable management of hypothermic victims could be devised in the future.

METHODS

This study used mature New Zeland White rabbits for the animal model to be tested. The rabbits were anesthetized with pentobarbitol at the onset of all experimental procedures to allow the induction of hypothermia. The animals were never permitted to return to a conscious state and all efforts were made prior to the experiments to ensure humane treatment. The rabbits were cooled externally via a water blanket and circulating water bath after the chest, back, and abdomen were shaved. Figure 1 shows the sequential protocol that was used. Femoral arteries (collapsed od approximately 1.2mm) were the blood vessels selected and the following experimental values were recorded from 1.8 X 10.6 mm helical strips:

1. Norepinephrine thresholds (The Grass Polygraph was set at 0.05 mv/cm after the original calibration was at 0.5 mv/cm where 500 mg = 25 mm).

2. Three minute norepinephrine dose-response curves to determine:

   a. Maximal tension developed with each dose of agonist.
   b. Contraction speed (time, sec, to reach 75% of maximal tension).

The bath contained physiological salt solution (PSS) which was aerated with 95% O_2 and 5% CO_2. The PSS had this composition in millimoles per liter: NaCl, 119; KCl, 4.7; KH_2PO_4, 1.18; M_gSO_4, 1.17; $NaHCO_3$, 14.9; dextrose, 5.5; sucrose, 50; $CaCl_2$, 1.6; and calcium disodium EDTA, 0.026. The dose-response curves and the norepinephrine thresholds were determined at the $\Delta l/lo = 0.5$ length. Here Δl is the increment in resting length above the initial length lo; lo is the length of the strip when it is subjected to the smallest resting tension which will keep it hanging straight in the bath.

RESULTS

Norepinephrine thresholds for both the hypothermic and normothermic derived tissues were determined at bath temperatures of 25°C and 37°C. The 37°C threshold determinations were made when: 1) the strips had equilibrated for 2 hours at that temperature and 2) after the strips had been in the bath for >

Figure 2

12 hours. Figure 2 shows the 25°C norepinephrine thresholds for both the hypothermic and normothermic derived tissue. In both instances, the thresholds were in the 10^{-6} g/ml and 10^{-7} g/ml ranges of agonist. The vast majority of the recordings for the hypothermic thresholds being at 10^{-7} g/ml , whereas the normothermic thresholds are more equally divided between the 10^{-6} g/ml and 10^{-7} g/ml levels. Figure 3 displays the thresholds after the strips had

Figure 3

equilibrated at 37°C for two hours. Both types of tissue display increased sensitivity to this agonist, but equally apparent is the distinctive separation of sensitivities. The hypothermic strips are, in most instances, from 10 to 100X more sensitive than the normothermic strips. However, as we see in figure 4, this augmented sensitivity appears to diminish after 12 hours. The dose-response curves of the hypothermic derived strips shift to the left of the normothermic derived during the period of augmented sensitivity (fig. 5).

Figure 4

Therefore, there would be greater contractile strength shown by the hypothermic derived tissue at the lower doses of agonist. This persisted to the 10^{-7} g/ml level. In figure 6 we see a comparison of contraction speeds between the hypothermic and normothermic obtained strips during the three different temperature trials. The hypothermic obtained strips are significantly slower ($P < 0.05$) than the controls when it is cold (25°C) and after it has been warm for > 12 hours. The only time it was not significantly slower, was during the period of enhanced norepinephrine sensitivity of the early warming period. Even though

Figure 5

Figure 6

the results show the slowest contractions when both tissues are cold, the fastest during the early warming period, and later an intermediate value after the >12 hour warming, significant differences could not be shown between the normothermic obtained strips at these different in vitro temperatures. There was however, a significant increase ($P < 0.05$) in contraction speed for the hypothermic obtained strips as they were first warmed. This difference did not last, and, after 12 warm in vitro hours, the contraction speed for the warm tissue was comparable to the cold. There were no significant differences in maximal contractility between the two sources of arterial strips. However, when the tissues were cold (25°C), they developed about 50% of the maximal tension possible ($P < 0.05$) as compared to when the strips had been warm (37°C) for two hours or more (fig 7).

Figure 7

DISCUSSION AND CONCLUSIONS

The arterial blood pressure reflex is a finely tuned negative feedback system involving arterial baroreceptors, cardiovascular control centers, and efferent descending pathways that can alter total peripheral resistance and cardiac output. This study has identified long-term hypothermic alterations of arterial reactivity and contractility that can profoundly modify the stability of this reflex. The afferent portion of this reflex would be changed at the level of the arterial baroreceptors due to the increased norepinephrine sensitivity, increased contractile strength at lower doses of agonist, and decreased rate of wall tension development. The sensitivity changes would likely lead to increased vasomotor tone under normal sympathetic innervation activity, therefore, the baroreceptors would be less sensitive to a given pressure load. Moreover, because of the decreased rate of tension development, these receptor structures could not adjust as rapidly to control center regulation. At the effector end of this reflex, the arteries would have a reduced radius under normal efferent discharge, thereby magnifying the total peripheral resistance and the arterial pressure. Hence, the cardiovascular control centers would be receiving sensory input from detector mechanisms that had been dulled and, at the same time, be sending efferent discharge to blood vessels with amplified reactivity. Synaptic control could not proceed accurately under these conditions, which probably accounts for the exaggerated fluctuations of blood pressure following hypothermia. Clinical intervention with vasoactive drugs might very well magnify these adverse conditions. The mechanism for these vascular wall modifications remains obscure. We have observed that they last for greater than twelve hours <u>in vitro</u> and probably longer <u>in vivo</u>. The fact that they persist so long in a physiological salt solution would suggest some

enduring structural change occured in vivo which involves macromolecules. The active force-length relationship appears unaltered because we see comparable contractility between the hypothermic and normothermic derived blood vessel strips. However, the velocity of contraction is slowed. Therefore, the number of cross-bridges being formed must be equal, but the cycling rate has probably been altered (18). This could be due to the level of cytoplasmic calcium present that is serving as the activator. It has been suggested that activator calcium fluxes are dependent on the source of the calcium i.e. transmembrane, sarcoplasmic reticulum, and mitochondria. Perhaps under these conditions, a more slowly exchanging site, i.e. the mitochondria, becomes the primary calcium source (3). It could also be a function of augmented calcium efflux or calcium uptake into intracellular organelles. Reduced rate of calcium influx or organelle discharge could also explain the observed results. These alterations in calcium homeostasis could also be implicated in contributing to the observed non-deviant supersensitivity to norepinephrine. Norepinephrine is presumed to start contractions in part by mobilizing calcium from intracellular pools. A change in pool size or source would therefore contribute to the supersentivity by increasing the availability of activating calcium. Another potential mechanism would call for changes in the translocation of calcium at the plasma membrane which in turn would change the electrophysiological properties to permit a reduction in the depolarization necessary for threshold response (8,9,11). Alterations to receptors such as changing either the affinity or density of receptors for drugs has long been suggested as a cause for non-deviant supersensitivity (4). However, current evidence is either lacking or does not support these possibilities for this tissue. The results of this study establish prolonged alterations to vascular smooth

muscle contractility and reactivity due to hypothermia. However, it offers sparse evidence for mechanism. Additional studies are in order that not only test this evidence with other species, but also provide definitive tests of cellular and subcellular calcium homeostasis and receptor affinity and/or density.

DISCLAIMER

The views, opinions, and/or findings contained in this report are that of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation.

Investigators adhered to the "Guides for the Care and Use of Laboratory Animals, " as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Footnotes

to page 1 - Dr. N.R. Bandick is presently a Professor of Biology at Western Oregon State College, Monmouth, OR 97361.

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NORMOTHERMIC RABBITS

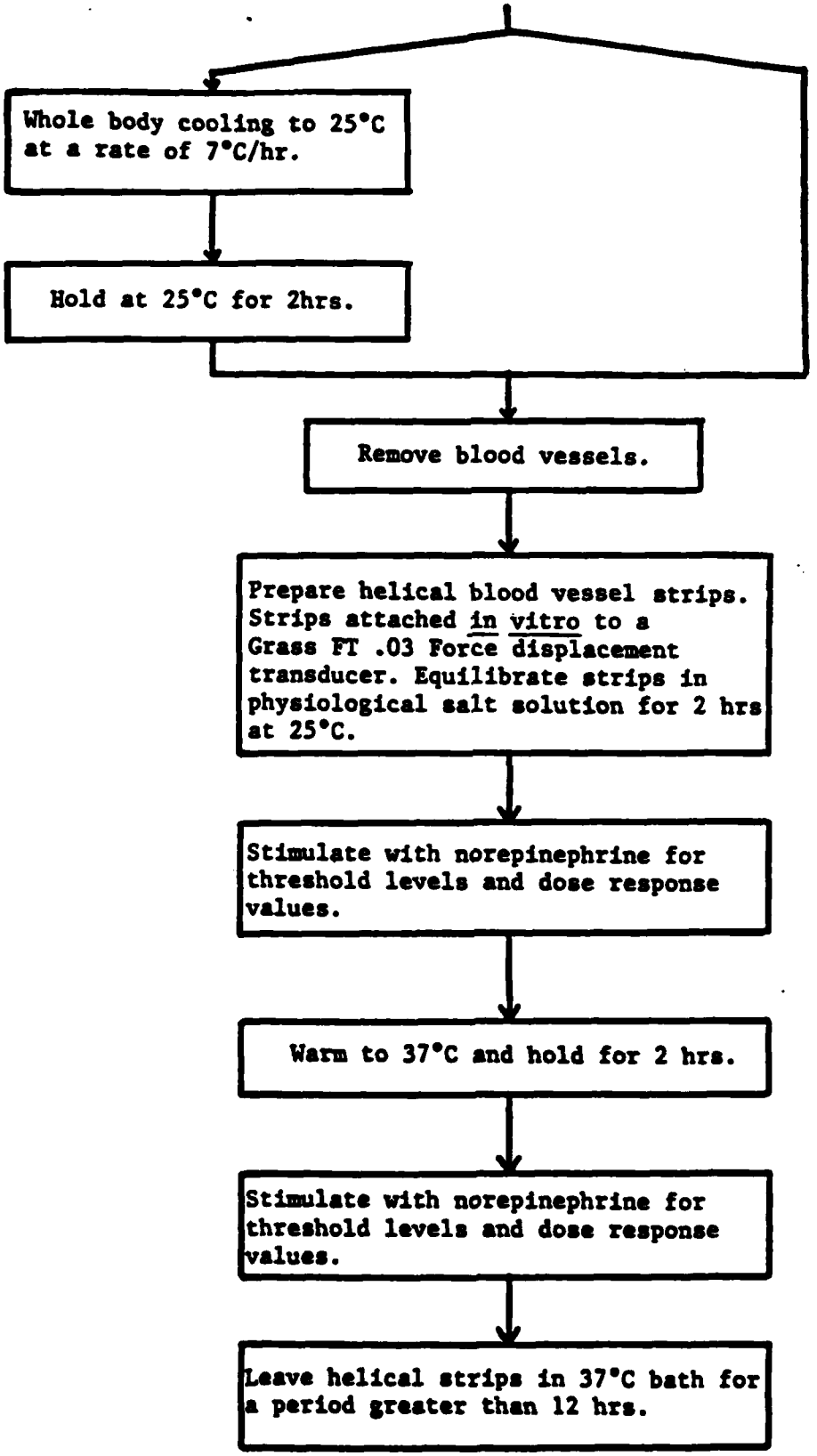


Fig 1

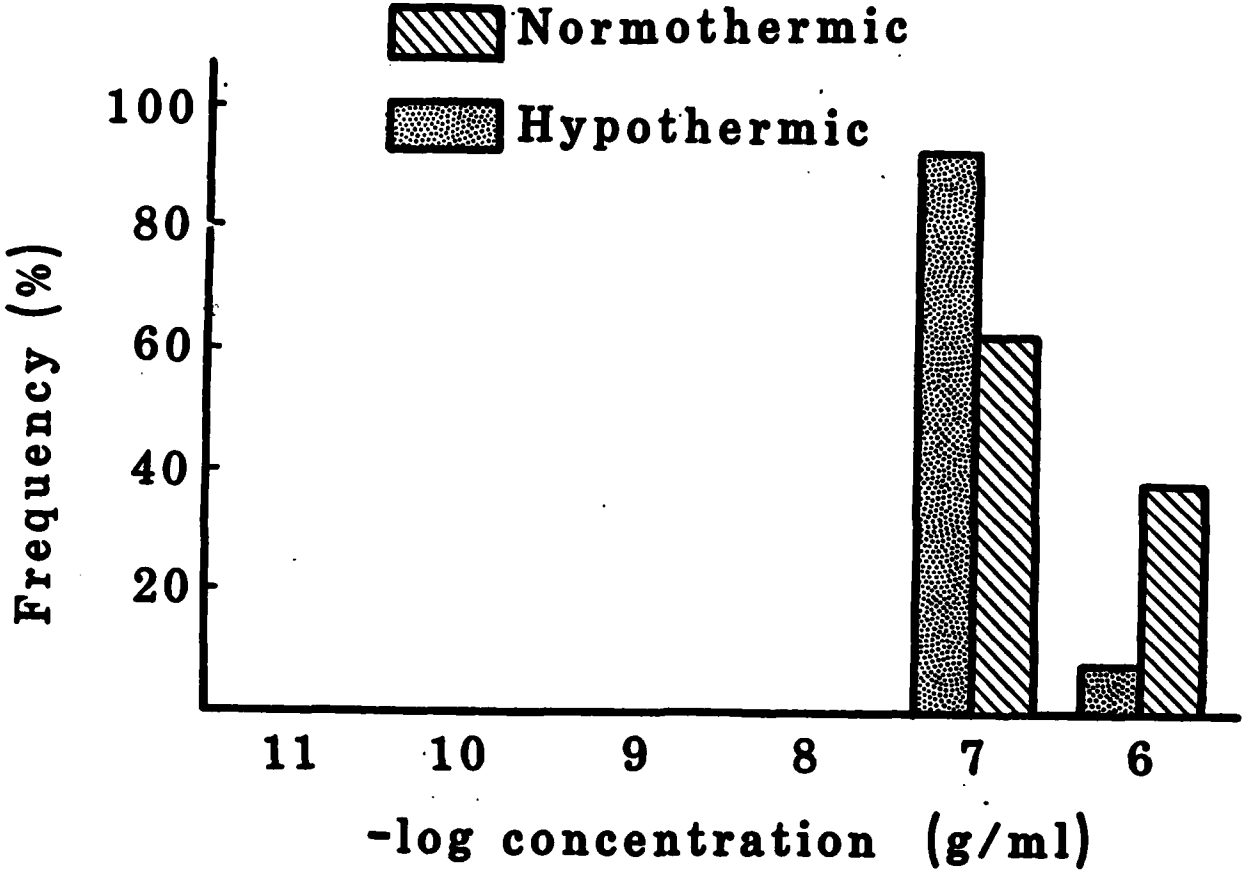

Normothermic
Hypothermic
100
80
60
40
20
Frequency (%)
11
10
9
8
7
6
-log concentration (g/ml)

fig 3

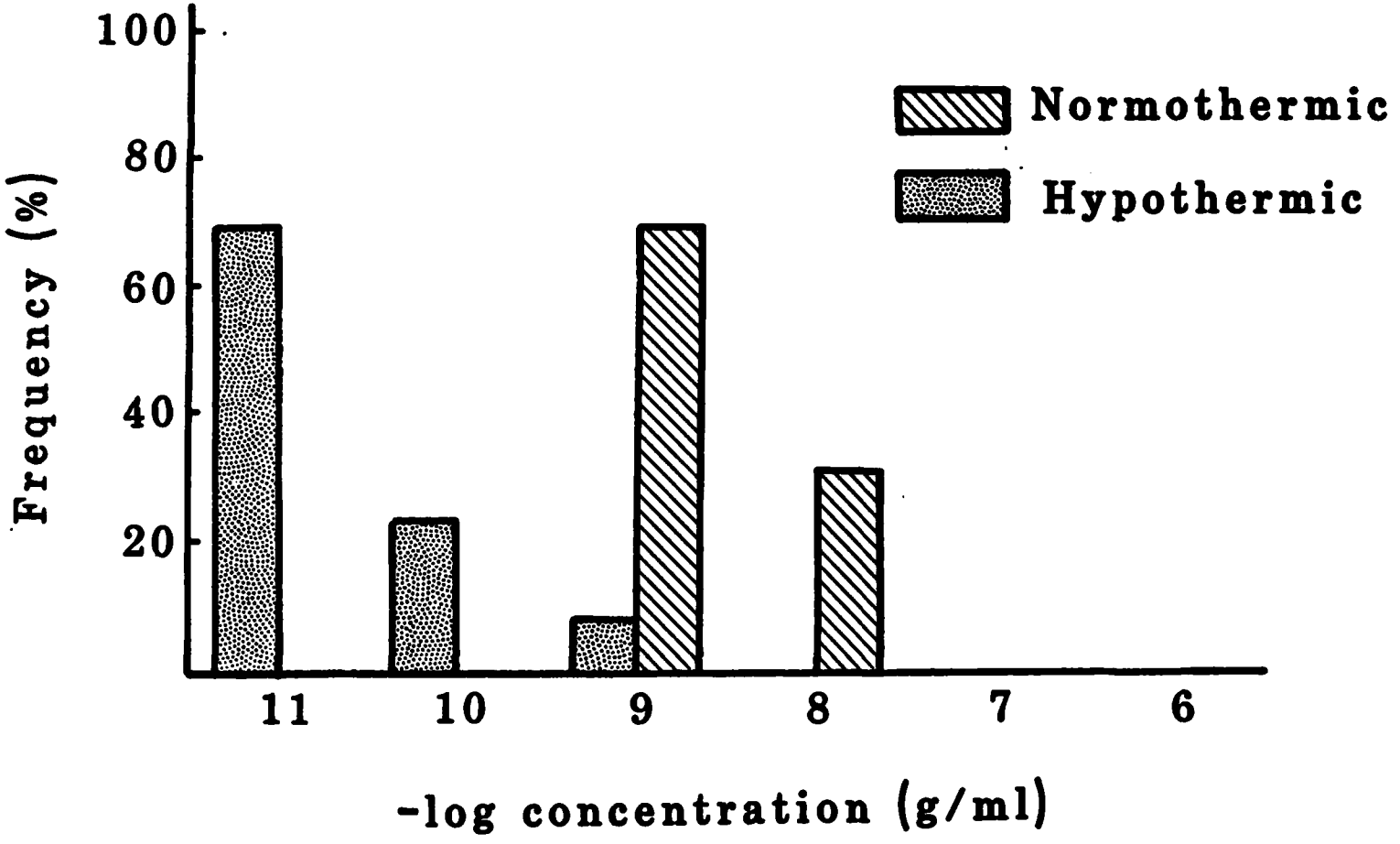

100
80
60
40
20
Frequency (%)
Normothermic
Hypothermic
11
10
9
8
7
6
-log concentration (g/ml)

Fig 4

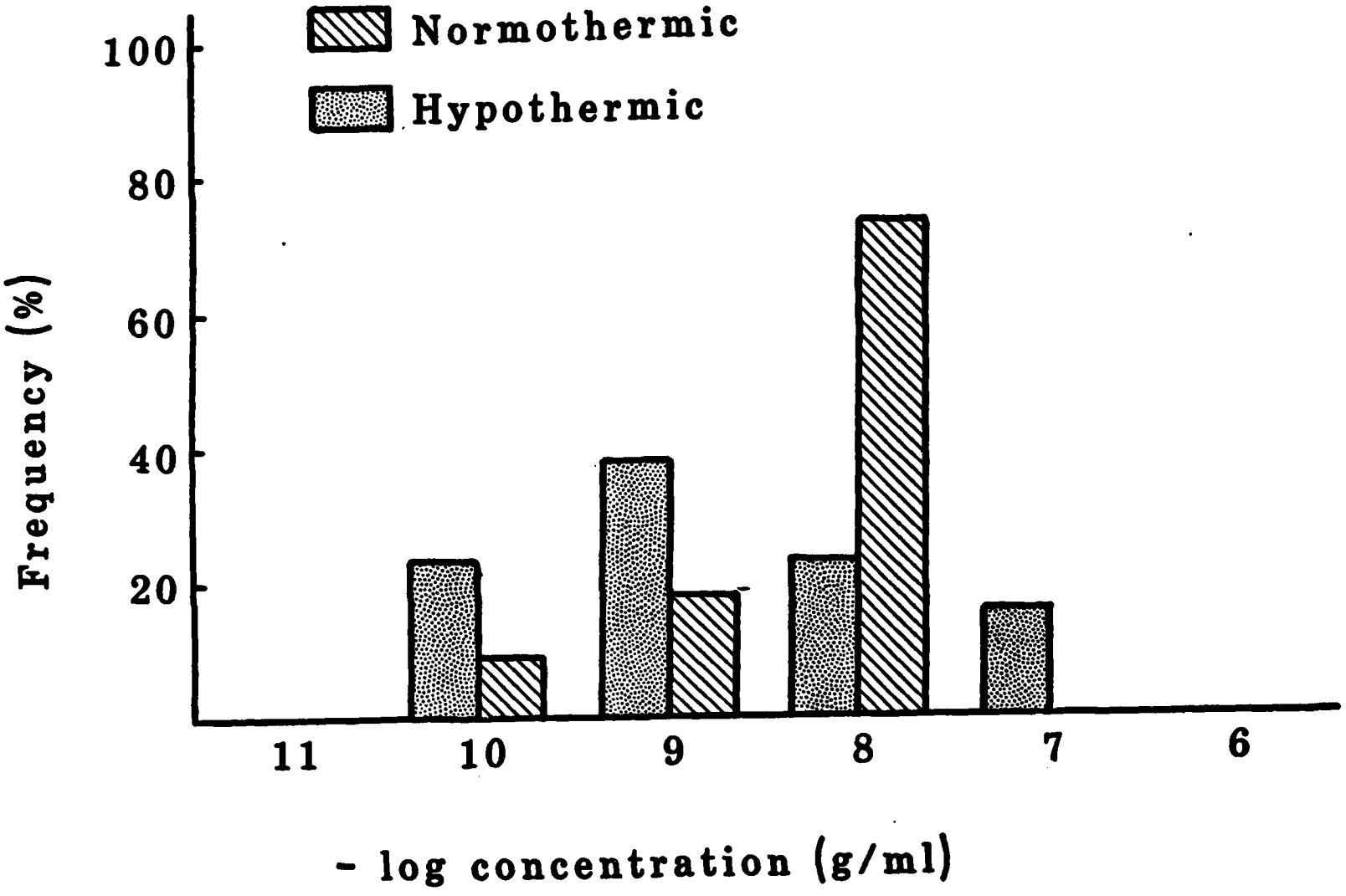
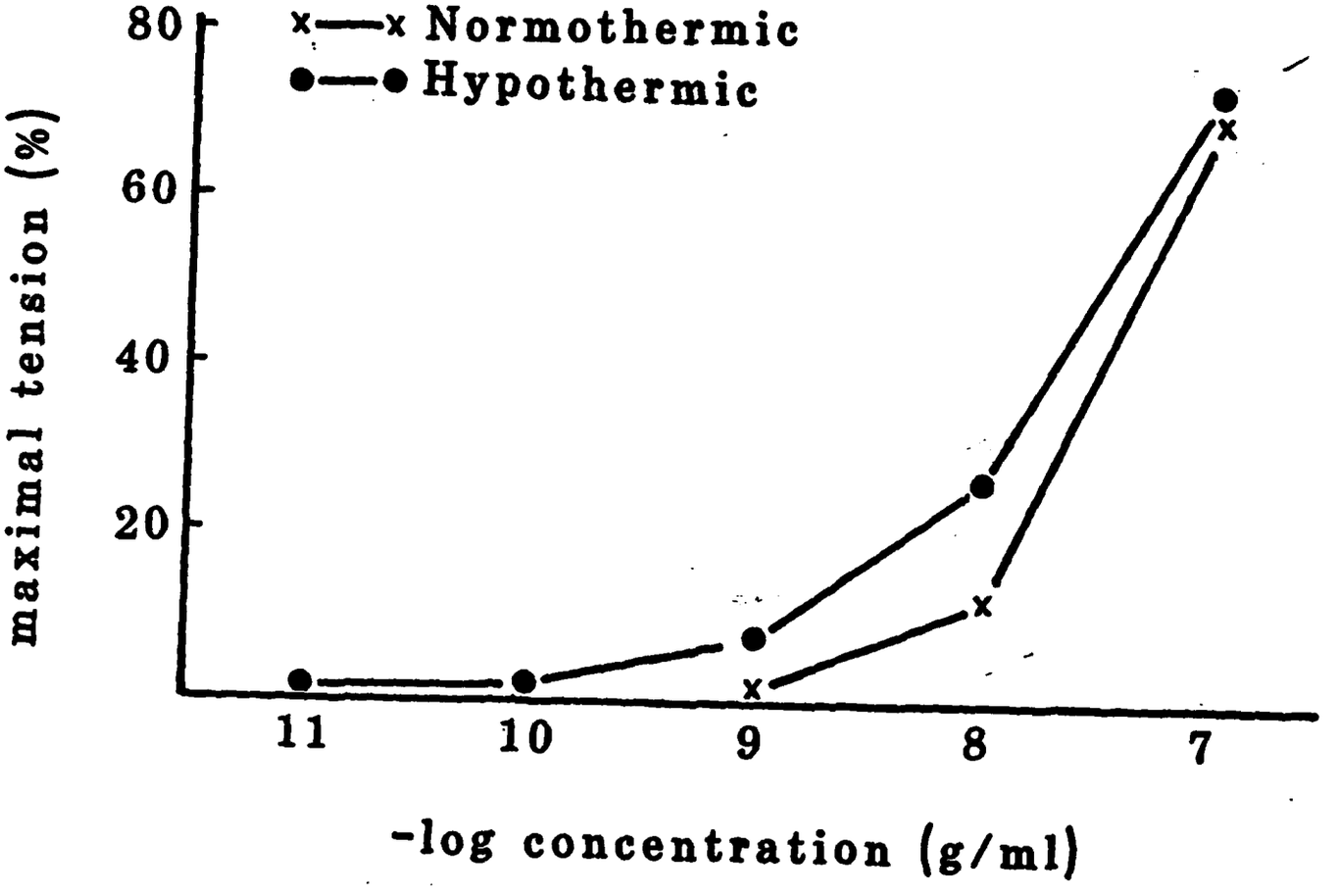
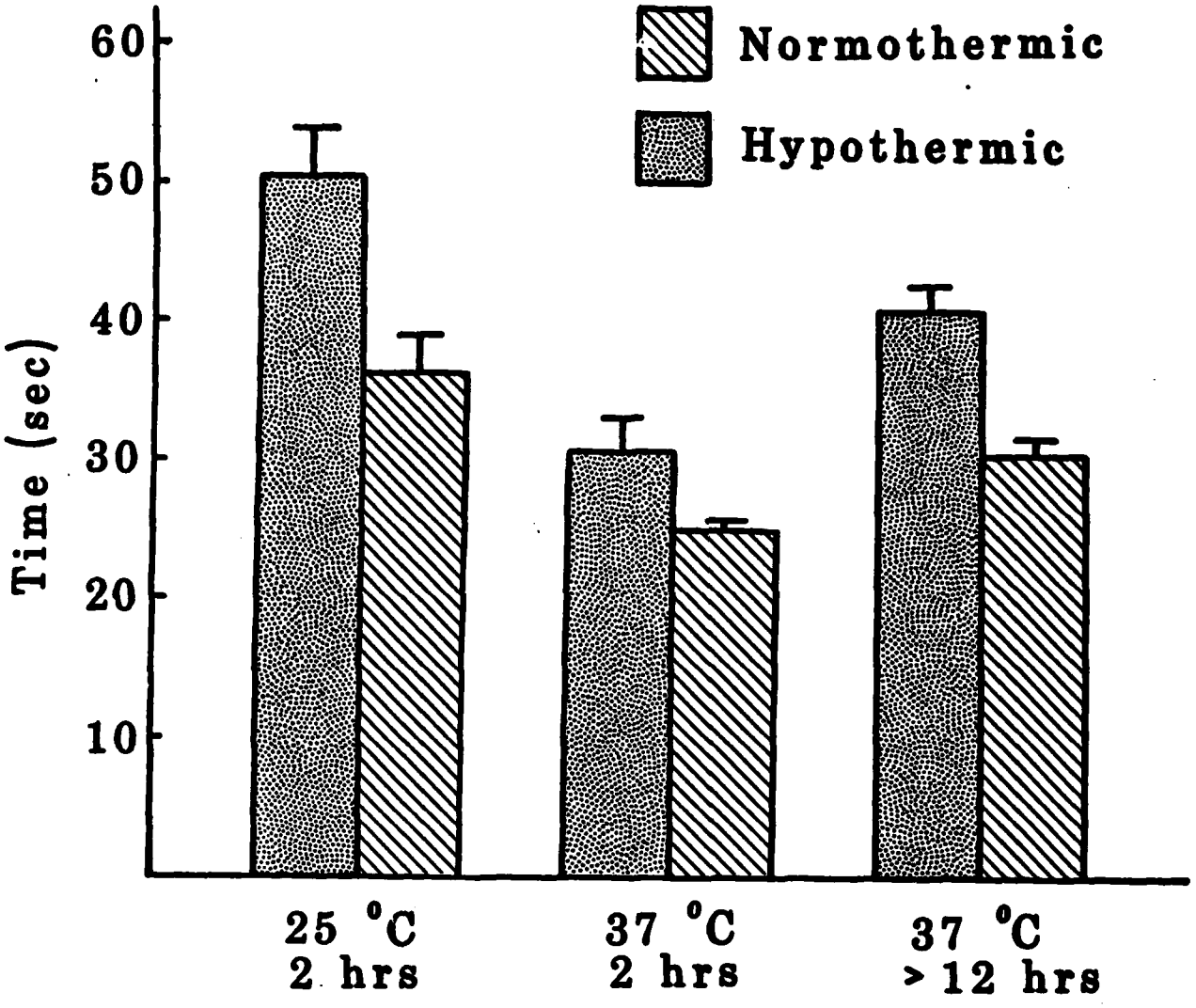
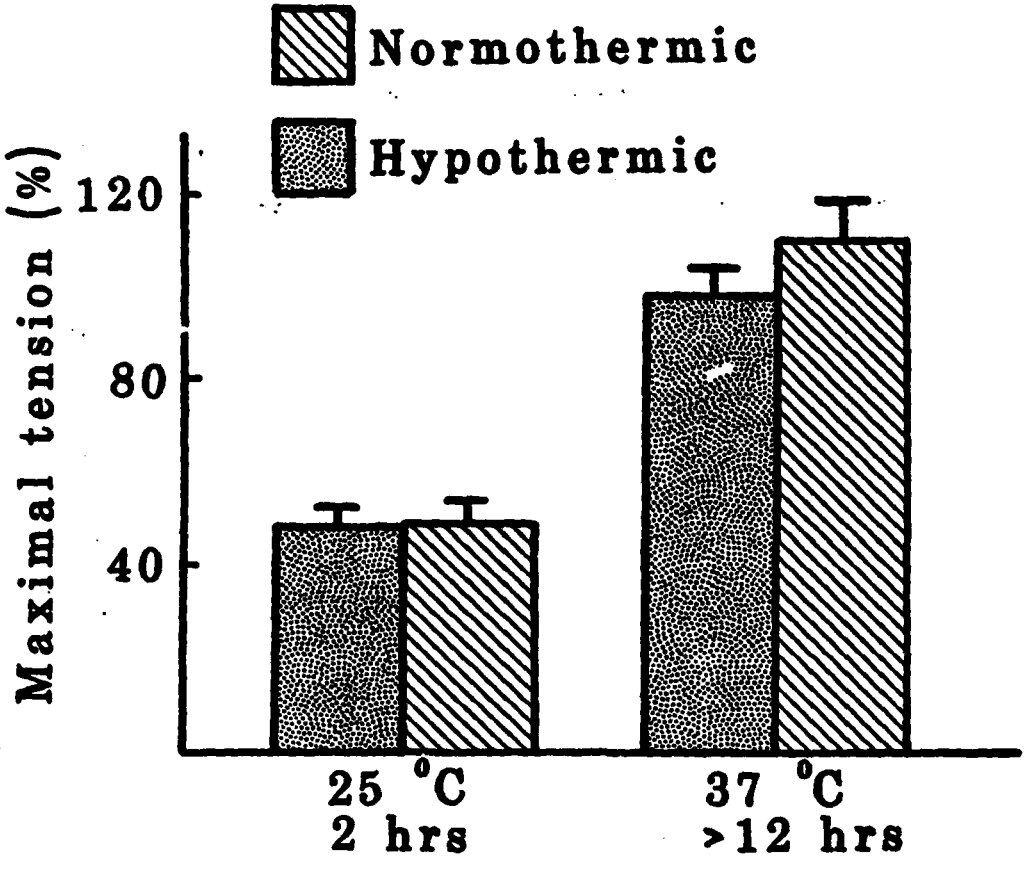

Normothermic
Hypothermic
100
80
60
40
20
Frequency (%)
11
10
9
8
7
6
- log concentration (g/ml)

Fig 5


x—x Normothermic
●—● Hypothermic
maximal tension (%)
80
60
40
20
11
10
9
8
7
-log concentration (g/ml)


Normothermic
Hypothermic
Time (sec)
60
50
40
30
20
10
25 °C
2 hrs
37 °C
2 hrs
37 °C
> 12 hrs


Normothermic
Hypothermic
Maximal tension (%)
120
80
40
25 °C
2 hrs
37 °C
>12 hrs

END

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